

Award Number: DAMD17-02-1-0484

TITLE: Gene Expression Analysis of Breast Cancer Progression

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REPORT DATE: July 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20031121 046

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2003	3. REPORT TYPE AND DATES COVERED Annual (17 Jun 02 - 16 Jun 03)	
4. TITLE AND SUBTITLE Gene Expression Analysis of Breast Cancer Progression			5. FUNDING NUMBERS DAMD17-02-1-0484	
6. AUTHOR(S) William L Gerald, M.D., Ph.D.			8. PERFORMING ORGANIZATION REPORT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Sloan-Kettering Institute for Cancer Research New York, New York 10021 E-Mail: Geraldw@mskcc.org				
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Breast cancer (BC) is a heterogeneous disease with varying clinical behavior, and response to therapy that cannot be predicted based on clinical and pathologic classifications. It is the primary goal of our research to identify and characterize biological pathways and individual molecular components that play a primary role in BC development and progression. In order to identify genes, gene expression profiles and molecular pathways associated with metastatic BC we have performed genome-wide gene expression analysis of a large number of breast cancer samples. Both unsupervised and supervised analyses are being used to identify genes differentially expressed among samples. Hierarchical clustering showed that most samples grouped according to estrogen receptor status. In addition, matched primary carcinomas and lymph node metastases tended to pair demonstrating marked conservation of molecular phenotype within patients. Formal statistical testing is being used to identify genes with marked changes in expression during progression. Lymph node metastases in particular showed significant decreases in the expression of many genes corresponding to extracellular matrix proteins and proteases when compared to matched primaries. Further expression changes in a variety of genes were associated with distant metastases. Immunohistochemistry and in situ hybridization are being used to validate and extend findings.				
14. SUBJECT TERMS			15. NUMBER OF PAGES 6	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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INTRODUCTION

Breast cancer (BC) is a heterogeneous disease with varying clinical behavior and response to therapy that cannot be predicted based on existing clinical and pathologic classifications. This has led to an intense effort to understand the biology of BC and a search for genes and gene products that play a major role in tumor development and progression. A comprehensive analysis of gene expression can provide crucial clues concerning the intrinsic biology of a cancer and ultimately contribute to diagnostic decisions and therapies tailored to an individual patient. New, high-throughput mRNA analysis platforms, such as DNA microarrays, allow comprehensive measurement of gene expression and can produce large data sets with the potential to provide novel insights into biology at the molecular level. Our studies are designed to identify gene expression profiles that are associated with tumor progression and can be used for discrimination of clinically relevant subgroups of BC. An understanding of the mechanisms that drive progression of BC will provide biomarkers for diagnosis, risk stratification and therapeutic targets that could have an enormous impact on the care of these patients. The specific aims of our project are: 1) To identify the genes, gene expression profiles and molecular pathways associated with metastatic BC using microarray based, gene expression analysis and comparison of concurrent primary and metastatic tumors within the same patients. 2) To identify gene expression differences associated with clinical outcome by comparison of comprehensive expression profiles from stage and histology matched primary BCs in patients with long term recurrence-free survival and patients that die of metastatic disease.

BODY

Task 1 To identify the genes, gene expression profiles and molecular pathways associated with metastatic breast cancer using microarray based, gene expression analysis and comparison of concurrent primary and metastatic tumors within the same patients

- a. Evaluation and selection of tumor cases to be used (months 1-6)
- b. Microdissection of frozen tissue, RNA preparation and analysis. (months 3-9)
- c. Microarray screen and analysis of data (months 6-12)
- d. Characterization of differentially expressed genes (months 9-24)
- e. Design and optimization of methods for molecular testing in additional samples (quantitative RT-PCR, in situ hybridization, etc). (months 18-30)
- f. Testing of additional cases and data analysis (months 24-36)

We have completed all tasks originally proposed for the first year of funding. Specifically we have identified and processed all tissue samples planned for specific aims 1 and 2. For specific aim 1, RNA has been isolated from 8 non-neoplastic mammary epithelium, 23 paired primary and lymph node metastasis within the same patients, 12 metastatic breast carcinomas from different sites and 3 non-neoplastic lymph nodes. Labeled cRNA target from these samples has been subjected to gene expression analysis using oligonucleotides microarrays with features for over 33000 genes/ESTs. Both unsupervised and supervised analyses are being used to identify genes differentially expressed among samples.

Hierarchical clustering of the gene expression data showed that most samples grouped according to estrogen receptor status (ER). In addition, the matched primary carcinomas and lymph node metastases have global expression profiles more similar to each other than to other breast cancers and tended to cluster together (Figure 1).. Formal statistical testing identified

genes that had marked changes in expression during progression. In particular lymph node metastases showed significant decreases in the expression of many genes corresponding to extracellular matrix proteins and proteases when compared to matched primaries. Very few genes were overexpressed in lymph node metastases compared to the corresponding primary. Further expression changes in a variety of genes were associated with distant metastases. Immunohistochemistry and in situ hybridization are being used to validate and extend findings.

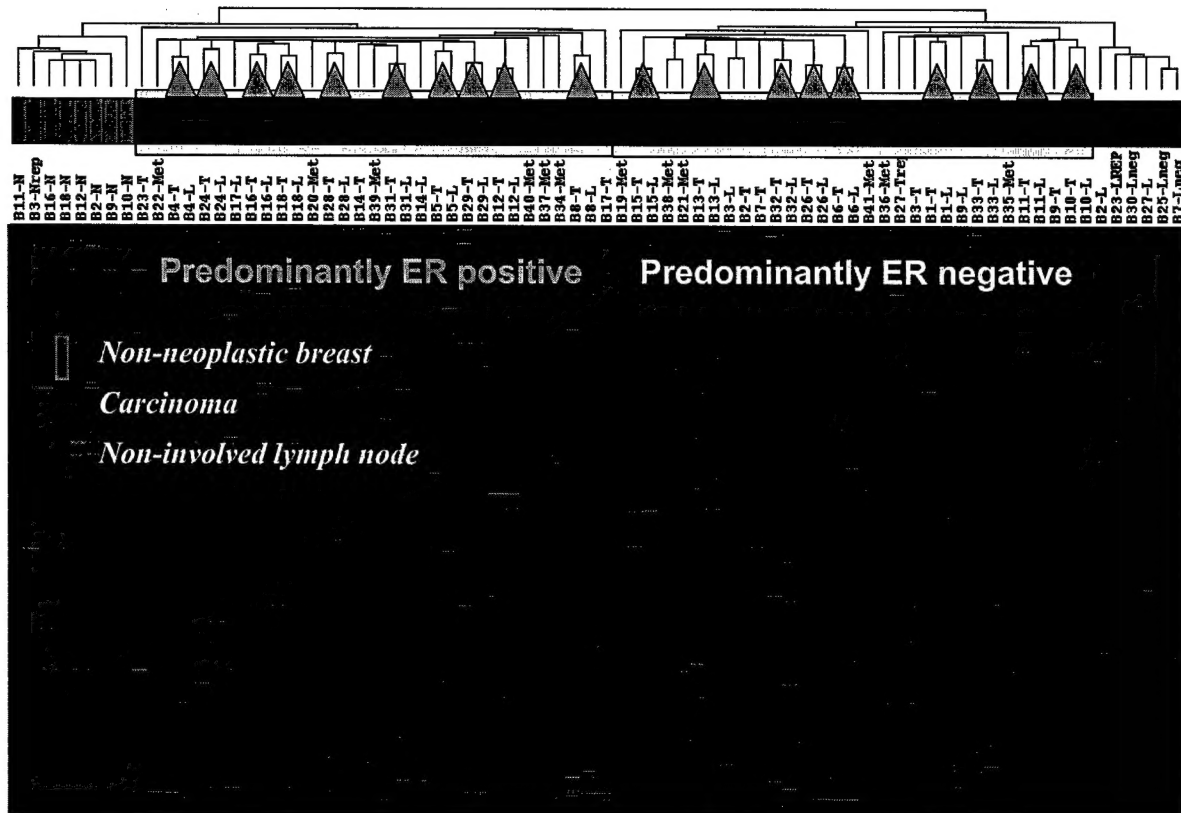


Figure 1. Hierarchical clustering of samples based on 10,000 U133 probe sets with greatest variation. Note clustering of ER positive (yellow background) and ER negative samples (light blue background) and pairing of matched primary and lymph node metastasis from the same patient (green triangles). Sample types are indicated by colored boxes.

Task 2 To identify gene expression differences associated with clinical outcome by comparison of comprehensive expression profiles from stage and histology matched primary breast cancers in patients with long term recurrence-free survival and patients that die of metastatic disease.

- Evaluation and selection of tumor cases to be used (months 3-9)
- Microdissection of frozen tissue, RNA preparation and analysis. (months 6-12)
- Microarray screen and analysis of data (months 9-15)
- Characterization of differentially expressed genes (months 12-24)
- Design and optimization of methods for molecular testing in additional samples (quantitative RT-PCR, in situ hybridization, etc). (months 18-30)

f. Testing of additional cases and data analysis (months 24-36)

For specific aim 2, we have identified, processed and performed expression analysis for 72 early stage primary breast cancers with appropriate outcome data that are matched for stage and treatment. Forty-six have no recurrence with a median follow-up of about 7 years and 26 have had a distant recurrence. We have performed an initial evaluation and found that the data quality is good and are beginning more formal analysis. These data will be further explored in year 2 of funding.

KEY RESEARCH ACCOMPLISHMENTS

- 1) Evaluation and selection of tumor cases to be used for specific aims 1 and 2
- 2) Microdissection of frozen tissue, RNA preparation and analysis of all samples.
- 3) Microarray based gene expression analysis of all samples.
- 4) Initial analysis of data from specific aim 1 and identification of differentially expressed genes.
- 5) Validation of differential expression at the RNA and protein level in progress for select genes.

REPORTABLE OUTCOMES

Comprehensive gene expression analysis of paired primary breast carcinomas and lymph node metastases. MCV. Donaton, D Giri, A Olshen, K Panageas, S Levcovici, P Lal, E Brogi, C Hudis, K VanZee, L Tan, W Gerald. Abstract presentation American Association of Cancer Research, 2003.

Gene expression differences between paired primary and metastatic breast carcinomas. D Giri, M Donaton, A Olshen, K Panageas, P Lal, S Levcovici, E Brogi, C Hudis, K VanZee, L Tan, W Gerald. Abstract presentation United States and Canadian Academy of Pathology, 2003.

CONCLUSIONS

Comprehensive gene expression analysis of archived breast cancer samples is feasible. Molecular subgroups of breast carcinoma identified by gene expression analysis are strongly influenced by the ER status of the tumor. The gene expression profiles of paired primary and metastatic breast carcinomas are remarkably similar and the differences observed appear to reflect different microenvironments and tissue specific responses to tumor growth. Taken together, these results suggest that molecular features of breast carcinomas metastatic to lymph nodes are largely present in the primary tumor and might have been acquired early in tumorigenesis. Further analysis of primary tumors from patients with differing outcomes will help to define genes associated with progression.

REFERENCES

None

APPENDICES

None